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(54) **METHOD FOR CULTIVATING PLANT RESISTANT TO GRAY LEAF SPOT**

(57)   The present invention discloses a method for cultivating a plant resistant to gray leaf spot. The proteins provided by the present invention are obtained from corn and named as ZMPK protein, and are the proteins represented by seq. 2, seq. 4, seq. 7 or seq. 9 in the sequence list. Nucleic acid molecules encoding the ZMPK proteins are also within the scope of the present invention. The invention further sets forth a method for preparing a transgenic plant, comprising the step of: introducing the nucleic acid molecules into a starting plant to obtain the transgenic plant with reduced resistance to gray leaf spot. The invention further sets forth a method for preparing a transgenic plant, comprising the step of: knocking out or inhibiting the expression of the nucleic acid molecules in a starting plant to obtain the transgenic plant with increased resistance to gray leaf spot. The present invention is of great application value to the breeding of corn resistant to gray leaf spot.

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Description

<u>**Technical Field**</u>

5   **[0001]**   The present invention belongs to the field of biotechnology, and specifically relates to a method for cultivating plants resistant to gray leaf spot; more specifically, it relates to a method for genetically improving plants by expressing a *ZmPK* gene to obtain plants resistant to gray leaf spot.

<u>**Background Art**</u>

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[0002]   Corn gray leaf spot is a worldwide fungal disease of corn leaves. It was first discovered in Alexandria County, Illinois, USA in 1925, and seriously affected the yield of corn. In China, the corn gray leaf spot first occurred in Dandong City, Liaoning Province in 1991. After that, it was reported in Jilin, Hebei, Yunnan and other regions. In recent years, gray leaf spot has become one of the main leaf diseases in corn production in China, especially in the southwest corn

15   producing areas. The occurrence of corn gray leaf spot generally causes a 10-30% reduction in corn production, and in severe cases, it can reach 60-80% or even no harvest, which severely affects the production of corn in China.
[0003]   Current researchers believe that there are two main pathogens causing corn gray leaf spot: *Cercospora zeae-maydis* (*Czm*) and *Cercospora zeina* (*Cz*). For a long time, domestic researchers believed that the pathogen of corn gray leaf spot in China was *Czm.* Liu et al. took samples in the Yunnan area and analyzed the morphology, pathogenicity,

20   ITS sequence and histone H3 gene sequence of the microorganism and found that the pathogen of corn gray leaf spot in the Yunnan area in China is *Cz*. The disease spots first appeared on the lower leaves, and the symptoms were most obvious on the leaves. In the early stage of onset, the disease spots were water-stained faded-green spots, and then expanded to grayish-brown color spots, which were approximately rectangular and parallel to the veins of the leaves. When the disease is severe, the disease spots expand and spread, causing the leaves to wither. The use of fungicides

25   and other chemical control methods is not effective. Breeding varieties resistant to gray leaf spot is the most economical and effective way to control this disease.

<u>**Summary of the Invention**</u>

30   **[0004]**   The present invention provides a method for cultivating plants resistant to gray leaf spot.
[0005]   The present invention provides a protein, which is obtained from corn, and named as ZmPK protein, which is as follows: (a1), or (a2), or (a3), or (a4), or (a5), or (a6), or (a7), or (a8):

(a1) a protein represented by SEQ ID NO: 2 in the sequence listing;
35   (a2) a protein represented by SEQ ID NO: 4 in the sequence listing;
(a3) a protein represented by SEQ ID NO: 7 in the sequence listing;
(a4) a protein represented by SEQ ID NO: 9 in the sequence listing;
(a5) a fusion protein obtained by attaching a tag to an N-terminus or/and a C-terminus of the protein in any one of (a1) to (a4);
40   (a6) a protein comprising the following three segments from N-terminus to C-terminus: the protein in any one of (a1) to (a4), a connecting peptide, and an EGFP protein;
(a7) a protein related to plant gray leaf spot resistance obtained by substituting and/or deleting and/or adding one or a plurality of amino acid residues to the protein in any one of (a1) to (a6); and
(a8) a protein related to plant gray leaf spot resistance obtained from corn and having a homology of 90%, 91%,
45   92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the protein in any one of (a1) to (a4).

[0006]   The tags are as shown in Table 1 below.

Table 1 Tag sequences

Tag	Residues	Sequence
Poly-Arg	5 to 6 (typically 5)	RRRR
Poly-His	2 to 10 (typically 6)	HHHHHH
FLAG	8	DYKDDDDK
Strep-tag II	8	WSHPQFEK
c-myc	10	EQKLISEEDL

(continued)

Tag	Residues	Sequence
HA	9	YPYDVPDYA
EGFP	239	Sequence 15

[0007] The EGFP protein is specifically shown in Sequence 15 in the sequence Listing. The connecting peptide can be specifically as shown in Sequence 19 in the sequence listing.

[0008] The protein can be synthesized artificially, or its encoding gene can be synthesized first, and then the protein can be obtained by biological expression.

[0009] The nucleic acid molecule encoding the ZmPK protein also falls within the scope of protection of the present invention.

[0010] The nucleic acid molecule is any one of the following (b1) to (b15):

(b1) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1618 in SEQ ID NO: 3 in the sequence listing;

(b2) a DNA molecule represented by SEQ ID NO: 3 in the sequence listing;

(b3) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1624 in SEQ ID NO: 5 in the sequence listing;

(b4) a DNA molecule represented by SEQ ID NO: 5 in the sequence listing;

(b5) a DNA molecule represented by SEQ ID NO: 1 in the sequence listing;

(b6) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1618 in SEQ ID NO: 8 in the sequence listing;

(b7) a DNA molecule represented by SEQ ID NO: 8 in the sequence listing;

(b8) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1624 in SEQ ID NO: 10 in the sequence listing;

(b9) a DNA molecule represented by SEQ ID NO: 10 in the sequence listing;

(b10) a DNA molecule represented by SEQ ID NO: 6 in the sequence listing;

(b11) a DNA molecule represented by SEQ ID NO: 12 in the sequence listing;

(b12) a DNA molecule represented by SEQ ID NO: 13 in the sequence listing;

(b13) a DNA molecule represented by SEQ ID NO: 14 in the sequence listing;

(b14) a DNA molecule that is derived from corn, has a homology of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with any one of (b1) to (b13), and encodes the protein;

(b15) a DNA molecule that hybridizes to any one of (b1) to (b13) under a stringent condition, and encodes the protein.

[0011] The stringent condition mentioned above is as follows: in a solution of 2×SSC, 0.1% SDS, hybridizing is performed at 68°C, and the membrane is washed twice for 5 min each time, and then hybridizing is performed again in a solution of 0.5×SSC, 0.1% SDS at 68°C, and the membrane is then washed twice for 15 min each time.

[0012] An expression cassette, recombinant vector or recombinant microorganism containing the nucleic acid molecule also falls within the scope of protection of the present invention.

[0013] The existing expression vectors can be used to construct a recombinant expression vector containing the nucleic acid molecule. When using the nucleic acid molecule to construct a recombinant expression vector, any enhanced, constitutive, tissue-specific or inducible promoter can be added before its transcription initiation nucleotide, and they can be used alone or in combination with other plant promoters that can be used in combination. In addition, when using the nucleic acid molecule to construct a recombinant expression vector, enhancers, including translation enhancers or transcription enhancers, can also be used. These enhancer regions can be ATG start codons or adjacent region start codons, etc., but they must be in the same reading frame with the coding sequence in order to ensure correct translation of the entire sequence. The sources of the translation control signals and initiation codons are extensive, and they can be natural or synthetic. The translation initiation region can be derived from a transcription initiation region or a structural gene. In order to facilitate the identification and screening of transgenic plants or transgenic microorganisms, the expression vectors used herein can be processed. For example, gene expressing enzymes or luminescent compounds that can produce color changes in plants or microorganisms, resistant antibiotic markers or chemical reagent resistant marker genes, etc. can be added herein. Considering the safety of the transgenes, it is possible to directly screen transformed plants or microorganisms by phenotype without adding any selectable marker genes.

[0014] The recombinant expression vector may specifically be a recombinant plasmid obtained by inserting the double-stranded DNA molecule shown in Sequence 12 in the sequence listing into the multiple cloning site (for example, the

*Bam*HI site) of the pCAMBIA3301 vector.

[0015] The recombinant expression vector may specifically be a recombinant plasmid obtained by inserting the nucleic acid molecule into the multiple cloning site (for example, the XcmI restriction site) of the pBCXUN vector.

[0016] The present invention further sets forth the application of the ZmPK protein, which is the following (c1) or (c2): (c1) to regulate the resistance of a plant to gray leaf spot; and (c2) to reduce the disease resistance of a plant to gray leaf spot.

[0017] The present invention further sets forth the application of the nucleic acid molecule, which is the following (d1) or (d2): (d1) to cultivate a transgenic plant with altered resistance to gray leaf spot; and (d2) to cultivate a transgenic plant with reduced resistance to gray leaf spot.

[0018] The application of the nucleic acid molecule further includes using the nucleic acid molecule as a target to reduce the expression amount of the nucleic acid molecule. The implementation methods include, but are not limited to: RNAi interference, gene knockout, etc. The implementation methods also include: insertion, deletion or editing of the promoter region, and promoter interchange. The methods for the target may also include, but are not limited to: using editing or mutant alleles with lower expression or weaker activity, etc.

[0019] The present invention further sets forth an application of a substance for inhibiting an activity of the ZmPK protein in a plant and/or for reducing an abundance of the ZmPK protein in a plant to enhance the disease resistance of the plant to gray leaf spot.

[0020] The present invention further sets forth an application of a substance for inhibiting a transcription of the nucleic acid molecule and/or for inhibiting an expression of the nucleic acid molecule and/or for gene editing of the nucleic acid molecule to enhance the disease resistance of the plant to gray leaf spot. The "substance for gene editing of the nucleic acid molecule" may specifically be any interference vector described later or any gene editing vector described later.

[0021] The present invention further sets forth a method for preparing a transgenic plant, comprising the steps of: introducing the nucleic acid molecule into a starting plant to obtain a transgenic plant with reduced gray leaf spot resistance. The nucleic acid molecule can be specifically introduced into the starting plant through any one of the above-mentioned recombinant expression vectors. The recombinant expression vector carrying the nucleic acid molecule can be transformed into the starting plant by conventional biological methods such as Ti plasmid, Ri plasmid, plant virus vector, direct DNA transformation, microinjection, electrical conduction, and agrobacterium mediation. By crossing the transgenic plants with existing corn varieties (including single crosses and multiple crosses, such as three consecutive crosses), the obtained transgenic progeny plants are also transgenic plants with reduced gray leaf spot resistance. The existing corn variety may specifically be a corn inbred line Q11.

[0022] The present invention further sets forth a plant breeding method, comprising the following steps: increasing a content and/or activity of the ZmPK protein in a target plant, thereby reducing the gray leaf spot resistance of the target plant.

[0023] The present invention further sets forth a plant breeding method, comprising the following steps: inhibiting an expression of the nucleic acid molecule in a starting plant to obtain a transgenic plant with increased gray leaf spot resistance. Inhibiting the expression of the nucleic acid molecule in the starting plant can be specifically achieved by means of introducing an interference vector. The interference vector may specifically be the following recombinant plasmid: a recombinant plasmid having a forward fragment, a spacer fragment and a reverse fragment; the spacer segment is used to space the forward segment and the reverse segment; the forward segment and the reverse segment are in a reverse complementary relationship; the forward fragment is shown in Sequence 11 in the sequence listing. The interference vector may specifically be the following recombinant plasmid: a recombinant plasmid obtained by using the pGreen-HY104 vector as a starting vector, and inserting forward fragments and reverse fragments into different multiple cloning sites; the forward segment and the reverse segment are in a reverse complementary relationship; and the forward fragment is shown in Sequence 11 in the sequence listing. The interference vector may specifically be the following recombinant plasmid: an RNAi interference vector obtained by using the pGreen-HY104 vector as the starting vector, inserting a forward fragment between the *Bam*HI and *Xba*I restriction sites, and inserting a reverse fragment between the *Hind*III and *EcoR*I restriction sites; the forward fragment and the reverse fragment are in a reverse complementary relationship; and the forward fragment is shown in Sequence 11 in the sequence listing. The interference vector can be transformed into the starting plant by conventional biological methods such as Ti plasmid, Ri plasmid, plant virus vector, direct DNA transformation, microinjection, electric conduction, agrobacterium mediation and the like. By crossing the transgenic plants with existing corn varieties (including single crosses and multiple crosses, such as three consecutive crosses), the obtained transgenic progeny plants are also transgenic plants with reduced gray leaf spot resistance. The existing corn variety may specifically be a corn inbred line Q11.

[0024] The present invention further sets forth a plant breeding method, comprising the following steps: reducing a content and/or activity of the protein ZmPK in a target plant, thereby increasing the disease resistance of the target plant to gray leaf spot.

[0025] The present invention further sets forth a plant breeding method, comprising the steps of: performing gene editing (causing a frameshift mutation in the specific gene) on a specific gene in a genome of a starting plant to increase

the gray leaf spot resistance of the target plant; and the specific gene encoding the ZmPK protein.

[0026] The gene editing is specifically realized by the Cas9 technology.

[0027] The gene editing is specifically realized by two sgRNAs and the Cas9 protein, in which the target sequence binding region of one sgRNA is shown in Sequence 17 of the sequence listing, and the target sequence binding region of the other sgRNA is shown in Sequence 18 of the sequence listing.

[0028] The gene editing is specifically realized by introducing a gene editing vector. The gene editing vector may specifically be the following recombinant plasmid: a recombinant plasmid obtained by inserting the double-stranded DNA molecule shown in Sequence 16 in the sequence listing into the *Bsa*I restriction site of the pBUE411 vector.

[0029] Any of the above-mentioned plants is a dicotyledonous plant or a monocotyledonous plant. The monocotyledonous plant may be a gramineous plant. The gramineous plant may be a plant of the genus Zea. The Zea plant may specifically be corn, such as corn inbred line B73 or corn inbred line B73-329.

Any of the above gray leaf spots may specifically be gray leaf spot caused by *Cercospora cornae.*

Brief Description of the Drawings

[0030]

Fig. 1 is a schematic diagram of the structure of a pBCXUN vector.

Fig. 2 is the results of the identification of gene expression level in Example 2.

Fig. 3 is the results of the identification of disease resistance in Example 2.

Fig. 4 is a schematic diagram of the structure of an RNAi interference vector.

Fig. 5 is the results of the identification of gene expression level in Example 3.

Fig. 6 is the results of the identification of disease resistance in Example 3.

Fig. 7 is the results of the correlation between the expression level of the *ZmPK* gene and the level of gray leaf spot.

Fig. 8 is a schematic diagram of the structure of a pCAMBIA3301 vector.

Fig. 9 is the results of the identification of gene expression level in Example 5.

Fig. 10 is the results of the identification of disease resistance in Example 5.

Fig. 11 is a schematic diagram of the structure of recombinant plasmid E and recombinant plasmid F.

Fig. 12 is the results of the identification of gene expression level in Example 6.

Fig. 13 is the results of the identification of disease resistance in Example 6.

Fig. 14 is the sequencing results of two gene-edited plants.

Fig. 15 is the results of the identification of disease resistance in Example 7.

Description of the Preferred Embodiments

[0031] The following examples facilitate a better understanding of the present invention, but do not limit the present invention. The experimental methods in the following examples, unless otherwise specified, are all conventional methods. The experimental materials used in the following examples, unless otherwise specified, are all purchased from conventional biochemical reagent stores. The quantitative experiments in the following examples are all set to repeat the experiment three times, and the results are averaged.

[0032] The corn inbred line Y32 is a corn inbred line with high resistance to gray leaf spot of corn. The corn inbred line Y32 (line Y32) is described in the following documents: QTL mapping of resistance to gray leaf spot in maize, Yan Zhang ect., Theor Appl Genet DOI 10.1007/s00122-012-1954-z.

[0033] The corn inbred line Q11 is a corn inbred line highly susceptible to gray leaf spot. The corn inbred line Q11 (line Q11) is described in the following documents: QTL mapping of resistance to gray leaf spot in maize, Yan Zhang ect., Theor Appl Genet DOI 10.1007/s00122-012-1954-z.

[0034] The corn inbred line B73 (B73 inbred lines) is described in the following documents: The B73 maize genome: complexity, diversity, and dynamics, Patrick S. Schnable ect., Science (2009) 326:1112-1115. DOI: 10.1126/science. 1178534; The tin 1 gene retains the function of promoting tillering in maize, Zhang ect., Nature communictions (2019) 5608. DOI: 10.1038/s41467-019-134225-6.

[0035] The corn inbred line B73-329 (B73-329 inbred lines) is described in the following documents: A retrotransposon in an HKT1 family sodium transporter causes variation of leaf Na$^+$ exclusion and salt tolerance in maize, Ming Zhang ect., New Phytologist (2018) 217: 1161-1176 doi: 10.1111/nph.14882.

[0036] *Cercospora zeina* is described in the following documents: First Report of Gray Leaf Spot of Maize Caused by *Cercospora zeina* in China, Plant Disease/Vol. 97 No. 12.

[0037] The pBCXUN vector is described in the following documents: ZmHAK5 and ZmHAK1 function in K$^+$ uptake and distribution in maize under low K+ conditions. Journal of Intergrative Plant Biology (2018) doi:10.1111/jipb. 12756.

The schematic diagram of the structure of a pBCXUN vector is shown in Fig.1.

[0038] The pGreen-HY104 vector (vector pGreen-HY104) is described in the following documents: A maize wall-associated kinase confers quantitative resistance to head smut, Nature Genetics (2015) 47:151-157.

[0039] The pCAMBIA3301 vector (bivalent expression vector *pCAMBIA3301*) is described in the following documents: Pyramiding of nine transgenes in maize generates high-level resistance against necrotrophic maize pathogens.

[0040] The pBUE411 vector is described in the following documents: ZmCCT9 enhances maize adaptation to higher latitudes. Huang ect., PNAS (2018) 115(2): E334-E341 DOI: 10.1073/pnas.1718058115.

Example 1. Discovery of ZmPK protein and its encoding gene

[0041] The corn inbred line Y32 (as the donor parent) and the corn inbred line Q11 (as the recurrent parent) were used to construct the initial positioning population and the fine positioning population. The molecular markers located in the finely-located regions were used; the Y32BAC library of disease-resistant parents was screened by PCR. BAC clone fingerprint analysis was performed to construct BAC contigs covering the entire gene segment. The clone that could cover the least gene region was then selected for sequencing. A new gene was discovered through sequence alignment and expression analysis.

[0042] The *ZmPK* gene in the genomic DNA of the corn inbred line Y32 is shown in Sequence 1 in the sequence listing. There are two transcripts of the *ZmPK* gene. The first transcript encodes the protein shown in Sequence 2 in the sequence listing; the second transcript encodes the protein shown in Sequence 4 in the sequence listing. The open reading frame corresponding to the first transcript in the cDNA of the corn inbred line Y32 is shown in nucleotides 56-1618 in Sequence 3 in the sequence listing. The open reading frame corresponding to the second transcript in the cDNA of the corn inbred line Y32 is shown in nucleotides 56-1624 in Sequence 5 in the sequence listing.

[0043] The *ZmPK* gene in the genomic DNA of the three corn inbred lines (the three corn inbred lines refer to the corn inbred line B73, the corn inbred line B73-329 and the corn inbred line Q11, the same below) is as shown in Sequence 6 in the sequence listing. There are two transcripts of the *ZmPK* gene. The first transcript encodes the protein shown in Sequence 7 in the sequence listing. The second transcript encodes the protein shown in Sequence 9 in the sequence listing. The open reading frame corresponding to the first transcript of the cDNAs of the three corn inbred lines is shown in nucleotides 56-1618 in Sequence 8 in the sequence listing. The open reading frame corresponding to the second transcript of the cDNAs of the three corn inbred lines is shown in nucleotides 56-1624 in Sequence 10 in the sequence listing.

Example 2. Obtaining and identifying over-expression plants

I. Construction of recombinant expression vector

[0044]

1. Fresh leaves of corn inbred line Y32 were taken, total RNA was extracted, and cDNA was obtained by reverse transcription.

2. The cDNA obtained in step 1 was used as a template; a primer pair composed of ZmPK-OE-F and ZmPK-OE-R was used for PCR amplification to obtain a PCR amplification product.

ZmPK-OE-F: 5'-ATGGGCGCTTGCTTCTCCTC-3';
ZmPK-OE-R: 5' -TCACAGAGCCTGAGGGTTTGG-3' .

3. The pBCXUN vector was taken, cleaved with restriction enzyme XcmI, and the vector backbone was recovered.

4. The PCR amplification product obtained in step 2 was linked to the vector backbone obtained in step 3 to obtain a recombinant plasmid A and a recombinant plasmid B. According to the sequencing results, the structure of recombinant plasmid A was described as follows: at the XcmI restriction site of the pBCXUN vector, a DNA molecule represented by nucleotides 56-1618 in Sequence 3 in the sequence listing was inserted. According to the sequencing results, the structure of recombinant plasmid B was described as follows: at the XcmI restriction site of the pBCXUN vector, a DNA molecule represented by nucleotides 56-1624 in Sequence 5 in the sequence listing was inserted. Since there were two sequences in the template and the difference was only 6 nucleotides, and the recombinant plasmid A and the recombinant plasmid B were constructed in the same way and produced at the same time, they needed to be identified by sequencing. In practical applications, two exogenous fragments can also be directly synthesized and then inserted into the XcmI restriction site of the pBCXUN vector to obtain a recombinant plasmid A and a recombinant plasmid B.

5. Fresh leaves of corn inbred line Q11 were taken, total RNA was extracted, and a cDNA was obtained by reverse

transcription.

6. The cDNA obtained in step 5 was used as a template; a primer pair composed of ZmPK-OE-F and ZmPK-OE-R was used for PCR amplification to obtain a PCR amplification product.

7. The PCR amplification product obtained in step 6 was linked to the vector backbone obtained in step 3 to obtain a recombinant plasmid C. According to the sequencing results, the structure of recombinant plasmid C was described as follows: at the XcmI restriction site of the pBCXUN vector, a DNA molecule represented by nucleotides 56-1618 in Sequence 8 in the sequence listing was inserted.

II. Acquisition of an overexpression plant

[0045]

1. The recombinant plasmid was introduced into Agrobacterium EHA105 to obtain recombinant Agrobacterium.
2. The recombinant Agrobacterium obtained in step 1 was taken, and the Agrobacterium-mediated method was used to genetically transform the immature embryos of the corn inbred line B73-329 to obtain the T0 generation plants.
3. The T0 generation plants were identified by PCR.

[0046]   The PCR identification method was as follows: plant leaves were taken, genomic DNA was extracted, and a primer pair consisting of bar-F and bar-R was used for PCR amplification. If an amplified product of about 262 bps was obtained, the PCR identification result was positive, and the plant was a transgenic plant. If no amplified product was obtained, the PCR identification result was negative, and the plant was a non-transgenic plant.
bar-F: GAAGGCACGCAACGCCTACGA; bar-R: CCAGAAACCCACGTCATGCCA.

[0047]   The recombinant plasmid A was used for step two, and three randomly selected transgenic plants were named, namely YT1-1 plant, YT1-2 plant, and YT1-3 plant. The recombinant plasmid B was used to perform step two, and three randomly selected transgenic plants were named, namely YT2-1 plant, YT2-2 plant, and YT2-3 plant. The recombinant plasmid C was used for step two, and three randomly selected transgenic plants were named, namely QT1-1 plant, QT1-2 plant, and QT1-3 plant.

III. Obtaining backcrossed separated offspring

[0048]   The T0 generation plants were selfed, the seeds were harvested, and the seeds were then cultivated into plants, that is, the T1 generation plants. The transgenic plants were screened from the T1 generation plants. The PCR identification method was the same as 3 in step II. The T1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the F1 generation plants. The transgenic plants were screened from the F1 generation plants, and the PCR identification method was the same as 3 in step II. The F1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the BC_1F_1 generation plants. The transgenic plants and non-transgenic plants were screened from the $BC_1 F_1$ generation plants, and the PCR identification method was the same as 3 in step II. The $BC_1 F_1$ generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, BC_2F_1 generation plants. The transgenic plants and non-transgenic plants were screened from the BC_2F_1 generation plants, and the PCR identification method was the same as 3 in step II. The BC_2F_1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, BC_3F_1 generation plants. The transgenic plants and non-transgenic plants were screened from the BC_3F_1 generation plants, and the PCR identification method was the same as 3 in step II.

[0049]   The T0 generation plants were: YT1-1 plant, YT1-2 plant, YT1-3 plant, YT2-1 plant, YT2-2 plant, YT2-3 plant, QT1-1 plant, QT1-2 plant, and QT1-3 plant.

IV. Identification of plant disease resistance

[0050]   The tested plants were: BC_3F_1 transgenic plants and non-transgenic plants of YT1-1 plants, BC_3F_1 transgenic plants and non-transgenic plants of YT1-2 plants, BC_3F_1 transgenic plants and non-transgenic plants of YT1-3 plants, BC_1F_1 generation transgenic plants and non-transgenic plants of YT2-1 plants, BC_1F_1 generation transgenic plants and non-transgenic plants of YT2-2 plants, BC_1F_1 generation transgenic plants and non-transgenic plants of YT2-3 plants, BC_2F_1 generation transgenic plants and non-transgenic plants of QT1-1 plants, BC_1F_1 generation transgenic plants and non-transgenic plants of QT1-2 plants, and BC_3F_1 generation transgenic plants and non-transgenic plants of QT1-3 plants.

1. Identification of gene expression level

[0051] The total RNA from the leaves of the tested plants was extracted and reverse transcribed to obtain the cDNA. With the GAPDH gene as the internal reference gene, the relative expression level of the *ZmPK* gene was detected by qPCR. The results are shown in Fig 2.

[0052] Primer pair used to detect *ZmPK* gene:

ZmPK-F: GCGTTGCCGTCAAGCGCAT; ZmPK-R:
GCTCCATCACAATGTACACGT.

[0053] Primer pair used to detect GAPDH gene:

GAPDH-F: ATCAACGGCTTCGGAAGGAT; GAPDH-R:
CCGTGGACGGTGTCGTACTT.

2. Identification of disease resistance

[0054] The disease resistance identification was carried out in Shangzhuang Experimental Base of China Agricultural University.

[0055] The pathogen of gray spot disease: *Cercospora zeina.*

[0056] The test plants were cultured under normal conditions, and pathogenic bacteria were inoculated during the bell mouth stage, and then the normal culture was continued. After two weeks of pollination, the phenotypic investigation was carried out, and the disease index (DSI) was calculated in a graded investigation. The specific method of inoculating pathogenic bacteria (bacterial fluid filling method) was as follows: the spores of the gray spot disease-causing fungus were added in sterile water to obtain a spore suspension with a spore concentration of $2\text{-}4 \times 10^5$/mL, and a syringe was used to infuse the spore suspension into the corn bell mouth, and 5 ml were infused per corn plant.

[0057] The grading standard of the disease level (X represents the percentage of the diseased spot area to the leaf area):

Level 1 (assigned value was 0): X$\leq$5%;
Level 3 (assigned value was 0.25): 5%<X$\leq$10%;
Level 5 (assigned value was 0.5): 10%<X$\leq$30%;
Level 7 (assigned value was 0.75): 30%<X$\leq$70%;
Level 9 (assigned value was 1): 70%<X$\leq$100%.
Disease index (DSI) (%) = $\sum$ (assigned value corresponding to the disease level x the number of plants at this level) x 100/1 x total number of plants
The results are shown in Fig. 3. In Fig. 3, the parentheses indicate the number of plants, the number before the dividing line is the number of non-transgenic plants, and the number after the dividing line is the number of transgenic plants.

[0058] The results show that, compared with non-transgenic plants, the expression of *ZmPK* gene in transgenic plants was significantly higher, and the disease index of corresponding transgenic plants was also significantly higher.

Example 3. Obtaining and identifying plants in which the expression is inhibited

I. Obtaining plants in which the expression is inhibited

[0059]

1. The pGreen-HY104 vector was used as the starting vector, the forward fragment was inserted between the *BamH*I and *Xba*I restriction sites, and the reverse fragment was inserted between the *Hind*III and *EcoR*I restriction sites, so as to obtain an RNAi interference vector. The RNAi interference vector was verified by sequencing. The forward segment and the reverse segment were in a reverse complementary relationship. The forward fragment is shown in Sequence 11 in the sequence listing. The structure diagram of the RNAi interference vector is shown in Fig. 4.
2. The RNAi interference vector obtained in step 1 was introduced into Agrobacterium EHA105 to obtain recombinant Agrobacterium.
3. The recombinant Agrobacterium obtained in step 2 was taken, and the Agrobacterium-mediated method was used to genetically transform the immature embryos of the corn inbred line B73-329 to obtain the T0 generation plants.
4. The T0 generation plants were selfed, the seeds were harvested, and the seeds were then cultivated into plants,

that is, the T1 generation plants.

5. The T0 generation plants were identified by PCR.

[0060] The PCR identification method was the same as 3 in step II in Example 2.

[0061] Three randomly selected transgenic plants were named as RNAi#806 plants, RNAi#1065 plants, and RNAi#581 plants.

II. Obtaining backcrossed separate population

[0062] The PCR identification method was the same as 3 in step II in Example 2.

[0063] The T0 generation plants were selfed, the seeds were harvested, and the seeds were then cultivated into plants, that is, the T1 generation plants. The transgenic plants were screened from the T1 generation plants (PCR identification). The T1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the F1 generation plants. The transgenic plants were screened from the F1 generation plants (PCR identification). The F1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the BC_1F_1 generation plants. The transgenic plants and non-transgenic plants were screened from the BC_1F_1 generation plants (PCR identification). The BC_1F_1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, BC_2F_1 generation plants. The transgenic plants and non-transgenic plants were screened from the BC_2F_1 generation plants (PCR identification).

[0064] The T0 generation plants were: RNAi#806 plant, RNAi#1065 plant, and RNAi#581 plant.

III. Identification of plant disease resistance

[0065] The tested plants were: BC_2F_1 transgenic plants and non-transgenic plants of RNAi#806 plants, BC_2F_1 transgenic plants and non-transgenic plants of RNAi#1065 plants, and BC_2F_1 transgenic plants and non-transgenic plants of RNAi#581 plants.

1. Identification of gene expression level

[0066] The method was the same as 1 in step IV in Example 2.

[0067] The results are shown in Fig. 5.

2. Identification of disease resistance

[0068] The method was the same as 2 in step IV in Example 2.

[0069] The results are shown in Fig. 6. In Fig. 6, the parentheses indicate the number of plants, the number before the dividing line is the number of non-transgenic plants, and the number after the dividing line is the number of transgenic plants.

[0070] The results show that, compared with non-transgenic plants, the expression of *ZmPK* gene in transgenic plants was significantly reduced, and the disease index of the corresponding transgenic plants was significantly reduced.

Example 4. Analysis of the correlation between gene expression and disease resistance

[0071] The original parents of the positioning population were corn inbred line Y32 and corn inbred line Q11. Through continuous crossing and backcrossing, a high-generation backcrossing segregation population was constructed.

[0072] Three plants with disease levels of 1, 3, 5, 7, and 9 in the positioning population were randomly selected, and gene expression level identification (the method was the same as that of 1 in step IV in Example 2) and disease resistance identification (the method was the same as 2 in step IV in Example 2) were performed. The correlation between the expression of *ZmPK* gene and the level of gray leaf spot was calculated. The results are shown in Fig. 7. There was a significant positive correlation between the expression of *ZmPK* gene and the level of gray leaf spot. This further confirms the negative correlation between *ZmPK* gene expression and plant resistance to gray leaf spot.

Example 5. Obtaining and identifying complementary transgenic plants

I. Construction of recombinant expression vector

[0073] A recombinant plasmid was prepared. According to the sequencing results, the structure of the recombinant

plasmid was described as follows: the DNA molecule shown in Sequence 12 in the sequence list was inserted into the *BamH*I restriction site of the pCAMBIA3301 vector. Fig. 8 shows a schematic diagram of the structure of the pCAMBIA3301 vector.

II. Obtaining complementary transgenic plants

[0074]

1. The recombinant plasmid prepared in step I was introduced into Agrobacterium EHA105 to obtain recombinant Agrobacterium.
2. The recombinant Agrobacterium obtained in step 1 was taken, and the Agrobacterium-mediated method was used to genetically transform the immature embryos of the corn inbred line B73 to obtain the T0 generation plants.
3. The T0 generation plants were identified by PCR.

[0075]   The PCR identification method was the same as 3 in step II in Example 2.
[0076]   A randomly selected transgenic plant was named C#596 plant.

III. Obtaining backcrossed separated offspring

[0077]   The T0 generation plants were selfed, the seeds were harvested, and the seeds were then cultivated into plants, that is, the T1 generation plants. The transgenic plants were screened from the T1 generation plants. The PCR identification method was the same as 3 in step II. The T1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the F1 generation plants. The transgenic plants were screened from the F1 generation plants, and the PCR identification method was the same as 3 in step II. The F1 generation transgenic plant was used as the female parent, and the corn inbred line Q11 was used as the male parent. The seeds were cultivated into plants, that is, the BC_1F_1 generation plants.

IV. Identification of plant disease resistance

[0078]   The tested plants were: BC_1F_1 generation transgenic plants and non-transgenic plants of C#595 plants, and BC_1F_1 generation transgenic plants and non-transgenic plants of C#596 plants.

1. Identification of gene expression level

[0079]   The method was the same as 1 in step IV in Example 2.
[0080]   The results are shown in Fig. 9.

2. Identification of disease resistance

[0081]   The method was the same as 2 in step IV in Example 2.
[0082]   The results are shown in Fig. 10. In Fig. 10, the parentheses indicate the number of plants, the number before the dividing line is the number of non-transgenic plants, and the number after the dividing line is the number of transgenic plants.
[0083]   The results show that, compared with non-transgenic plants, the expression of *ZmPK* gene in transgenic plants was significantly higher, and the disease index of corresponding transgenic plants was also significantly higher.

Example 6 Obtaining and identifying pure lines of fusion overexpression plants 1. Construction of recombinant expression vector

[0084]   The recombinant plasmid E and recombinant plasmid F were prepared respectively. According to the sequencing results, the structure of recombinant plasmid E was described as follows: at the *Xcm*I restriction site of the pBCXUN vector, a DNA molecule shown in Sequence 13 of the sequence listing was inserted. According to the sequencing results, the structure of recombinant plasmid F was described as follows: at the *Xcm*I restriction site of the pBCXUN vector, a DNA molecule shown in Sequence 14 of the sequence listing was inserted. The schematic diagram of the structure of recombinant plasmid E and recombinant plasmid F is shown in Fig. 11.

II. Obtaining pure lines of fusion overexpression plants

[0085]

1. The recombinant plasmid was introduced into Agrobacterium EHA105 to obtain recombinant Agrobacterium.
2. The recombinant Agrobacterium obtained in step 1 was taken, and the Agrobacterium-mediated method was used to genetically transform the immature embryos of the corn inbred line B73 to obtain T0 generation plants.
3. The T0 generation plants were identified by PCR.

[0086] The PCR identification method was the same as 3 in step II of Example 2.

[0087] The recombinant plasmid E was used to carry out step II, and four randomly selected transgenic plants were named: Y1#349 plant, Y1#350 plant, Y1#351 plant, and Y1#354 plant. The recombinant plasmid F was used to carry out step II, and three randomly selected transgenic plants were named, namely Y2#731 plant, Y2#732 plant, and Y2#735 plant.

[0088] 4. The T0 generation plants were selfed and the seeds were harvested. The seeds were cultivated into plants, which were the T1 generation plants. The transgenic plants (identified by PCR) were screened from the T1 generation plants for selfing, and the seeds were harvested. The T2 generation plants were cultivated from the seeds into the plants. The transgenic plants (identified by PCR) from the T2 generation plants were screened for selfing, and the seeds were harvested. The seeds were cultivated to plants, that is, the T3 generation plants. The PCR identification method was the same as 3 in step II in Example 2. For a T2 generation plant, if the T3 generation plants obtained by selfing were all transgenic plants, the T2 generation plant was a homozygous transgenic plant. The offspring obtained by selfing of the homozygous transgenic plant was a homozygous transgenic line.

[0089] The following homozygous transgenic strains were obtained: Y1#349 strain, Y1#350 strain, Y1#351 strain, Y1#354 strain, Y2#731 strain, Y2#732 strain, and Y2#735 strain.

III. Identification of plant disease resistance

[0090] The tested plants were: T3 generation plants of Y1#349 line, T3 generation plants of Y1#350 line, T3 generation plants of Y1#351 line, T3 generation plants of Y1#354 line, T3 generation plants of Y2#731 line, T3 generation plants of Y2#732 line, T3 generation plants of Y2#735 line, and the corn inbred line B73 plants.

1. Identification of gene expression level

[0091] The method was the same as 1 in step IV in Example 2.

[0092] The results are shown in Fig. 12.

2. Identification of disease resistance

[0093] The method was the same as 2 in Step IV in Example 2.

[0094] The results are shown in Fig. 13. In Fig. 13, the parentheses indicate the number of plants.

[0095] The results show that compared with the corn inbred line B73 plants (control non-transgenic plants), the expression of *ZmPK* gene in the transgenic plants was significantly higher, and the disease index of the corresponding transgenic plants was also significantly higher.

Example 7 Obtaining and identifying gene-edited plants

1. Construction of gene editing vector

[0096] A gene editing vector was prepared, that is, a recombinant plasmid was obtained by: inserting the double-stranded DNA molecule shown in Sequence 16 in the sequence listing into the *Bsa*I restriction site of the pBUE411 vector. The recombinant plasmid was verified by sequencing. The gene editing vector encoded two sgRNAs, the target sequence binding region of one sgRNA is shown in Sequence 17 in the sequence listing, and the target sequence binding region of the other sgRNA is shown in Sequence 18 in the sequence listing. The gene editing vector contained the Cas9 gene and expressed the Cas9 protein.

II. Obtaining gene-edited plants

[0097]

1. The gene editing vector was introduced into Agrobacterium EHA105 to obtain recombinant Agrobacterium.
2. The recombinant Agrobacterium obtained in step 1 was taken, and the Agrobacterium-mediated method was used to genetically transform the immature embryos of the corn inbred line B73 to obtain T0 generation plants.
3. The plants with mutations in the target sequence were screened from the T0 generation plants.

[0098]   The specific method was as follows: the plant leaves were taken, the genomic DNA was extracted, a primer pair composed of F and R was used for PCR amplification, and the PCR amplification product was recovered and sequenced. The sequencing result was compared with the wild-type sequence (the wild-type sequence is shown in the 9599-10985 in Sequence 12 in the sequence listing), and plants with different sequences were screened.

    F: 5'-TTGAGGTCATTGTCTCAGCC-3';
    R: 5' -AGCAGCTTGGCAGCGTATG-3'.

[0099]   4. The T0 generation plants screened in step 3 were taken, and used for selfing and then harvested to obtain the seeds. The seeds were cultivated into plants, which were the T1 generation plants.
[0100]   5. The gene-edited plants were screened from T1 generation plants.

    (1) The plants were subjected to PCR identification with the bar gene in the gene editing vector as the target. PCR identification method was as follows: plant leaves were taken, genomic DNA was extracted, and the primer pair containing bar-F and bar-R was used for PCR amplification. If no amplified product was obtained, the PCR identification result was negative.
    (2) The plants with the target region of gene editing were identified as the target. The identification method was the same as step 3.

[0101]   If a plant is identified as negative by PCR in step (1), and the identification result in step (2) shows that it is different from the wild-type sequence and is homozygous (and the two chromosomes are identical), the plant is a gene-edited plant.
[0102]   Two gene-edited plants were obtained, named ZmPK-KO#1 plant and ZmPK-KO#2 plant, respectively.
[0103]   The sequencing results of step (2) of the two gene editing plants are shown in Fig. 14. Compared with B73, two nucleotides were missing in the ZmPK-KO#1 plant, and a nucleotide was inserted into the ZmPK-KO#2 plant. Both caused frameshift mutations.
[0104]   6. The gene-edited plants were taken, self-bred, and offspring plants were obtained, that is, the gene-edited plants.
[0105]   Two gene editing lines were obtained, and named ZmPK-KO#1 strain and ZmPK-KO#2 strain, respectively.

3. Identification of disease resistance of gene-edited plants

[0106]   The tested plants were: T2 generation plants of ZmPK-KO#1 line, T2 generation plants of ZmPK-KO#2 line, and corn inbred line B73 plants.
[0107]   The method was the same as 2 in step IV in Example 2.
[0108]   The results are shown in Fig. 15. In Fig. 15, the parentheses indicate the number of plants.
[0109]   Compared with corn inbred line B73 plants, the disease index of gene-edited plants was significantly reduced.

Industrial applicability

[0110]   The present invention provides a major gene *ZmPK* for resistance to gray leaf spot of corn. Through transgene complementation, overexpression, CRISPR knockout and RNAi interference experiments, the gene's anti-grey leaf spot function-negative regulation of gray leaf spot resistance has been proved. The present invention has great application value for the breeding of corn against gray leaf spot.

SEQUENCE LISTING

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Val Asp Glu Arg Arg Pro Ser Lys Glu Gly Asp Gly Lys Lys Arg Arg
                20   25   30

Arg Ala Ala Gly Ala Ser Pro Asp Ala Ala Ala Pro Val Arg Val Glu
            35   40   45

Phe Gly Tyr Glu Arg Asp Phe Glu Ala Arg Tyr Glu Val Gly Arg Leu
        50   55   60

Leu Gly His Gly Gln Phe Gly Tyr Thr Phe Ala Ala Thr Asp Arg Gly
65   70   75   80

Ser Gly Asp Arg Val Ala Val Lys Arg Ile Asp Lys Ala Lys Met Thr
                85   90   95

Arg Pro Val Ala Val Glu Asp Val Lys Arg Glu Val Lys Ile Leu Lys
            100   105   110

Ala Leu Lys Gly His Gln Asn Ile Val His Phe Tyr Asn Ala Phe Glu
            115   120   125

Asp Asp Ser Tyr Val Tyr Ile Val Met Glu Leu Cys Glu Gly Gly Glu
        130   135   140

Leu Leu Asp Arg Ile Leu Ala Lys Lys Asn Ser Arg Tyr Ser Glu Lys
145   150   155   160

Asp Ala Ala Val Val Val Arg Gln Met Leu Lys Val Ala Ala Glu Cys
                165   170   175

His Leu Arg Gly Leu Val His Arg Asp Met Lys Pro Glu Asn Phe Leu
            180   185   190

Phe Lys Ser Asn Lys Glu Asp Ser Pro Leu Lys Ala Thr Asp Phe Gly
            195   200   205

Leu Ser Asp Phe Ile Lys Pro Gly Lys Lys Phe His Asp Ile Val Gly
        210   215   220

Ser Ala Tyr Tyr Val Ala Pro Glu Val Leu Lys Arg Arg Ser Gly Pro

17

	225				230				235				240	

Glu	Ser	Asp	Val	Trp	Ser	Ile	Gly	Val	Ile	Thr	Tyr	Ile	Leu	Leu	Cys
			245				250				255			

Gly	Arg	Arg	Pro	Phe	Trp	Asp	Lys	Thr	Glu	Asp	Gly	Ile	Phe	Lys	Glu
		260				265				270				

Val	Leu	Arg	Asn	Lys	Pro	Asp	Phe	Arg	Lys	Arg	Pro	Trp	Ser	Ser	Ile
		275				280				285				

Ser	Pro	Gly	Ala	Lys	Asp	Phe	Val	Lys	Arg	Leu	Leu	Val	Lys	Asn	Pro
	290				295				300					

Arg	Ala	Arg	Leu	Thr	Ala	Ala	Gln	Ala	Leu	Ser	His	Pro	Trp	Val	Arg
305				310				315				320		

Glu	Gly	Gly	Glu	Ala	Ser	Asp	Ile	Pro	Val	Asp	Ile	Ser	Val	Leu	Ser
			325				330				335			

Asn	Met	Arg	Gln	Phe	Val	Lys	Tyr	Ser	Arg	Phe	Lys	Gln	Phe	Ala	Leu
		340				345				350				

Arg	Ala	Leu	Ala	Ser	Thr	Leu	Asn	Glu	Glu	Glu	Leu	Ser	Asp	Leu	Lys
	355				360				365					

Asp	Gln	Phe	Asp	Ala	Ile	Asp	Ile	Asp	Lys	Ser	Gly	Ser	Ile	Ser	Ile
	370				375				380					

Glu	Glu	Met	Arg	His	Ala	Leu	Ala	Lys	Asp	Leu	Pro	Trp	Arg	Leu	Lys
385				390				395				400		

Gly	Pro	Arg	Val	Leu	Glu	Ile	Ile	Gln	Ala	Ile	Asp	Ser	Asn	Thr	Asp
			405				410				415			

Gly	Leu	Val	Asp	Phe	Lys	Glu	Phe	Val	Ala	Ala	Thr	Leu	His	Ile	His
			420				425				430			

Gln	Met	Ala	Glu	Leu	Asp	Ser	Glu	Arg	Trp	Gly	Ile	Arg	Cys	Gln	Ala
	435				440				445					

Ala	Phe	Ser	Lys	Phe	Asp	Leu	Asp	Gly	Asp	Gly	Tyr	Ile	Thr	Pro	Glu
	450				455				460					

Glu	Leu	Arg	Met	His	Pro	Gly	Leu	Lys	Gly	Ser	Ile	Glu	Pro	Leu	Leu
465				470				475				480		

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Glu Glu Ala Asp Ile Asp Lys Asp Gly Lys Ile Ser Leu Ser Glu Phe
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Arg Lys Leu Leu Arg Thr Ala Ser Met Ser Asn Val Pro Ser Pro Arg
             500                 505                 510


Gly Pro Pro Asn Pro Gln Ala Leu
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<210>  3
<211>  1934
<212>  DNA
<213>  Zea mays L.

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ggaccgcgtt gccgtcaagc gcatcgacaa ggccaagatg acccgccctg ttgctgtgga    360

ggatgtgaaa agagaagtga agattcttaa agcacttaaa ggacatcaga atattgttca    420

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cggtgaacta ttagatcgga ttttggcaaa aaagaatagc cgctatagtg agaaagatgc    540

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tgttggaagt gcttactatg tcgcaccaga agtactaaaa cgacggtctg gtcctgagtc    780

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gaggccttgg tcaagcatca gcccaggtgc taaagatttt gttaaaggt tactagtgaa    960

gaatccaagg gccaggctaa cagctgctca agctctctca catccgtggg taagagaagg   1020

aggggaagca tccgatatcc ccgtcgacat atctgtgtta tcaaacatgc gtcagtttgt   1080

caagtacagc cgtttcaagc aattcgcgct tcgggctctg gcgagcaccc ttaacgagga   1140

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gccggaggaa ctcagaatgc accctgggtt gaagggatct atcgagccgc tgctggagga      1500

ggccgacatc gacaaagacg gcaagataag cctgtccgag ttccgcaagc tcctacggac      1560

agcgagcatg agcaacgtac ccagcccaag ggggccccca aaccctcagg ctctgtgaat      1620

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cagtgcagcc ccgagtgagt ttcgaagtaa atattcagtg ctttcttttt ctttccggaa      1800

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tggagaagtg agga                                                        1934
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<212>    PRT
<213>    Zea mays L.

<400>    4

Met Gly Ala Cys Phe Ser Ser Ala Ser Ala Ala Pro Ala Gly Ala Ala
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Val Asp Glu Arg Arg Pro Ser Lys Glu Gly Asp Gly Lys Lys Arg Arg
                20                  25                  30


Arg Ala Ala Gly Ala Ser Pro Asp Ala Ala Ala Pro Val Arg Val Glu
            35                  40                  45


Phe Gly Tyr Glu Arg Asp Phe Glu Ala Arg Tyr Glu Val Gly Arg Leu
        50                  55                  60


Leu Gly His Gly Gln Phe Gly Tyr Thr Phe Ala Ala Thr Asp Arg Gly
65                  70                  75                  80


Ser Gly Asp Arg Val Ala Val Lys Arg Ile Asp Lys Ala Lys Met Thr
                85                  90                  95


Arg Pro Val Ala Val Glu Asp Val Lys Arg Glu Val Lys Ile Leu Lys
                100                 105                 110


Ala Leu Lys Gly His Gln Asn Ile Val His Phe Tyr Asn Ala Phe Glu
            115                 120                 125


Asp Asp Ser Tyr Val Tyr Ile Val Met Glu Leu Cys Glu Gly Gly Glu
        130                 135                 140
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Leu Leu Asp Arg Ile Leu Ala Lys Lys Asn Ser Arg Tyr Ser Glu Lys
145   150   155   160

Asp Ala Ala Val Val Val Arg Gln Met Leu Lys Val Ala Ala Glu Cys
                165   170   175

His Leu Arg Gly Leu Val His Arg Asp Met Lys Pro Glu Asn Phe Leu
            180   185   190

Phe Lys Ser Asn Lys Glu Asp Ser Pro Leu Lys Ala Thr Asp Phe Gly
        195   200   205

Leu Ser Asp Phe Ile Lys Pro Gly Lys Lys Phe His Asp Ile Val Gly
    210   215   220

Ser Ala Tyr Tyr Val Ala Pro Glu Val Leu Lys Arg Arg Ser Gly Pro
225   230   235   240

Glu Ser Asp Val Trp Ser Ile Gly Val Ile Thr Tyr Ile Leu Leu Cys
                245   250   255

Gly Arg Arg Pro Phe Trp Asp Lys Thr Glu Asp Gly Ile Phe Lys Glu
            260   265   270

Val Leu Arg Asn Lys Pro Asp Phe Arg Lys Arg Pro Trp Ser Ser Ile
        275   280   285

Ser Pro Gly Ala Lys Asp Phe Val Lys Arg Leu Leu Val Lys Asn Pro
    290   295   300

Arg Ala Arg Leu Thr Ala Ala Gln Ala Leu Ser His Pro Trp Val Arg
305   310   315   320

Glu Gly Gly Glu Ala Ser Asp Ile Pro Val Asp Ile Ser Val Leu Ser
                325   330   335

Asn Met Arg Gln Phe Val Lys Tyr Ser Arg Phe Lys Gln Phe Ala Leu
            340   345   350

Arg Ala Leu Ala Ser Thr Leu Asn Glu Glu Glu Leu Ser Asp Leu Lys
        355   360   365

Asp Gln Phe Asp Ala Ile Asp Ile Asp Lys Ser Gly Ser Ile Ser Ile
    370   375   380

Glu Glu Met Arg His Ala Leu Ala Lys Asp Leu Pro Trp Arg Leu Lys

	385				390				395				400	

Gly Pro Arg Val Leu Glu Ile Ile Gln Ala Ile Asp Ser Asn Thr Asp
               405   410   415

Gly Leu Val Asp Phe Lys Glu Phe Val Ala Ala Thr Leu His Ile His
            420   425   430

Gln Met Ala Glu Leu Asp Ser Glu Arg Trp Gly Ile Arg Cys Gln Ala
     435   440   445

Ala Phe Ser Lys Phe Asp Leu Asp Gly Asp Gly Tyr Ile Thr Pro Glu
     450   455   460

Glu Leu Arg Met Val Gln His Pro Gly Leu Lys Gly Ser Ile Glu Pro
465   470   475   480

Leu Leu Glu Glu Ala Asp Ile Asp Lys Asp Gly Lys Ile Ser Leu Ser
            485   490   495

Glu Phe Arg Lys Leu Leu Arg Thr Ala Ser Met Ser Asn Val Pro Ser
            500   505   510

Pro Arg Gly Pro Pro Asn Pro Gln Ala Leu
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<212>  DNA
<213>  Zea mays L.

<400>  5
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tgcagtggta gtccgccaaa tgctcaaagt agctgctgaa tgccatctgc gtgggttagt     600

tcaccgagat atgaagcctg agaacttcct tttcaaatcg aacaaggagg attcaccact     660
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gaggccttgg tcaagcatca gcccaggtgc taaagatttt gttaaaaggt tactagtgaa   960

gaatccaagg gccaggctaa cagctgctca agctctctca catccgtggg taagagaagg   1020

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caagtacagc cgtttcaagc aattcgcgct tcgggctctg gcgagcaccc ttaacgagga   1140

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ccgtgtgctg gagattattc aagcaattga cagcaacact gatgggctcg tggacttcaa   1320

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tgtgtgcagt gcagccccga gtgagtttcg aagtaaatat tcagtgcttt ctttttcttt   1800

ccggaagagt gagaggtgga ggtcaaaatg gtaggcaaga ctcgccttct tctttccttt   1860

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<210>   6
<211>   6946
<212>   DNA
<213>   Zea mays L.

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ggggaccgcg ttgccgtcaa gcgcatcgac aaggccaagg tgagctgccg cctgcccccc   360

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ctactagcta ccagaaaccc actttatgca ggtgttcagt ttaataacac ccaccatctt    4440

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atcttatttt gtggtagatt gacagcaaca ctgatgggct cgtggacttc aaggagtttg    6000

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gtttctggtt tttagcatcc aggttatgtg tgcagtgcag ccccgagtga gtttcgaagt        6780

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<210> 7
<211> 520
<212> PRT
<213> Zea mays L.

<400> 7

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                20                  25                  30


Arg Ala Ala Gly Ala Ser Pro Asp Ala Ala Ala Pro Val Arg Val Glu
                35                  40                  45


Phe Gly Tyr Glu Arg Asp Phe Glu Ala Arg Tyr Glu Val Gly Arg Leu
        50                  55                  60


Leu Gly His Gly Gln Phe Gly Tyr Thr Phe Ala Ala Thr Asp Arg Gly
65                  70                  75                  80


Ser Gly Asp Arg Val Ala Val Lys Arg Ile Asp Lys Ala Lys Met Thr
                85                  90                  95
```

27

```
        Arg Pro Val Ala Val Glu Asp Val Lys Arg Glu Val Lys Ile Leu Lys
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        Ala Leu Lys Gly His Gln Asn Ile Val His Phe Tyr Asn Ala Phe Glu
                    115             120             125

        Asp Asp Ser Tyr Val Tyr Ile Val Met Glu Leu Cys Glu Gly Gly Glu
                    130             135             140

        Leu Leu Asp Arg Ile Leu Ala Lys Lys Asn Ser Arg Tyr Ser Glu Lys
        145             150             155             160

        Asp Ala Ala Val Val Val Arg Gln Met Leu Lys Val Ala Ala Glu Cys
                    165             170             175

        His Leu Arg Gly Leu Val His Arg Asp Met Lys Pro Glu Asn Phe Leu
                    180             185             190

        Phe Lys Ser Asn Lys Glu Asp Ser Pro Leu Lys Ala Thr Asp Phe Gly
                    195             200             205

        Leu Ser Asp Phe Ile Lys Pro Gly Lys Lys Phe His Asp Ile Val Gly
                    210             215             220

        Ser Ala Tyr Tyr Val Ala Pro Glu Val Leu Lys Arg Arg Ser Gly Pro
        225             230             235             240

        Glu Ser Asp Val Trp Ser Ile Gly Val Ile Thr Tyr Ile Leu Leu Cys
                    245             250             255

        Gly Arg Arg Pro Phe Trp Asp Lys Thr Glu Asp Gly Ile Phe Lys Glu
                    260             265             270

        Val Leu Arg Asn Lys Pro Asp Phe Arg Lys Arg Pro Trp Ser Ser Ile
                    275             280             285

        Ser Pro Gly Ala Lys Asp Phe Val Lys Arg Leu Leu Val Lys Asn Pro
                    290             295             300

        Arg Ala Arg Leu Thr Ala Ala Gln Ala Leu Ser His Pro Trp Val Arg
        305             310             315             320

        Glu Gly Gly Glu Ala Ser Asp Ile Pro Val Asp Ile Ser Val Leu Ser
                    325             330             335

        Asn Met Arg Gln Phe Val Lys Tyr Ser Arg Phe Lys Gln Phe Ala Leu
                    340             345             350
```

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Arg Ala Leu Ala Ser Thr Leu Asn Glu Glu Glu Leu Ser Asp Leu Lys
        355                 360                 365

Asp Gln Phe Asp Ala Ile Asp Ile Asp Lys Ser Gly Ser Ile Ser Ile
        370                 375                 380

Glu Glu Met Arg His Ala Leu Ala Lys Asp Leu Pro Trp Arg Leu Lys
385                 390                 395                 400

Gly Pro Arg Val Leu Glu Ile Ile Gln Ala Ile Asp Ser Asn Thr Asp
                405                 410                 415

Gly Leu Val Asp Phe Lys Glu Phe Val Ala Ala Thr Leu His Ile His
                420                 425                 430

Gln Met Ala Glu Leu Asp Ser Glu Arg Trp Gly Ile Arg Cys Gln Ala
        435                 440                 445

Ala Phe Ser Lys Phe Asp Leu Asp Gly Asp Gly Tyr Ile Thr Pro Glu
        450                 455                 460

Glu Leu Arg Met His Thr Gly Leu Lys Gly Ser Ile Glu Pro Leu Leu
465                 470                 475                 480

Glu Glu Ala Asp Ile Asp Lys Asp Gly Lys Ile Ser Leu Ser Glu Phe
                485                 490                 495

Arg Lys Leu Leu Arg Thr Ala Ser Met Ser Asn Val Pro Ser Pro Arg
        500                 505                 510

Gly Pro Pro Asn Pro Gln Ala Leu
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<211>   1936
<212>   DNA
<213>   Zea mays L.

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ggcgcccgtg cgcgtggagt cggctacga gagggacttc gaggcgcgct acgaggtcgg   240

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tgcagtggta gtccgccaaa tgctcaaagt agctgctgaa tgccatctgc gtgggttagt      600

tcaccgagat atgaagcctg agaacttcct tttcaaatcg aacaaggagg attcaccact      660

aaaggcgaca gattttggtt tgtcagattt cattaagcca gggaagaagt tccatgacat      720

tgttggaagt gcttactatg tcgcaccaga agtactaaaa cgacggtctg gtcctgagtc      780

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gaggccttgg tcaagcatca gcccaggtgc taaagatttt gttaaaaggt tactagtgaa      960

gaatccaagg gccaggctaa cagctgctca agctctctca tccgtgggg taagagaagg      1020

aggggaagca tccgatatcc ccgtcgacat atctgtgtta tcaaacatgc gtcagtttgt      1080

caagtacagc cgtttcaagc aattcgcgct tcgggctctg gcgagcaccc ttaacgagga      1140

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gagtgagagg tggaggtcaa aatggtaggc aagactcgcc ttcttcttc ctttacactg      1860

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<210> 9
<211> 522
<212> PRT
<213> Zea mays L.

<400> 9

Met Gly Ala Cys Phe Ser Ser Ala Ser Ala Ala Pro Ala Gly Ala Ala

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                  20                  25              30

          Arg Ala Ala Gly Ala Ser Pro Asp Ala Ala Ala Pro Val Arg Val Glu
 10               35                  40              45

          Phe Gly Tyr Glu Arg Asp Phe Glu Ala Arg Tyr Glu Val Gly Arg Leu
                  50                  55              60

 15       Leu Gly His Gly Gln Phe Gly Tyr Thr Phe Ala Ala Thr Asp Arg Gly
          65                  70              75                  80

          Ser Gly Asp Arg Val Ala Val Lys Arg Ile Asp Lys Ala Lys Met Thr
 20                   85                  90                  95

          Arg Pro Val Ala Val Glu Asp Val Lys Arg Glu Val Lys Ile Leu Lys
                  100                 105                 110

 25       Ala Leu Lys Gly His Gln Asn Ile Val His Phe Tyr Asn Ala Phe Glu
                  115                 120                 125

          Asp Asp Ser Tyr Val Tyr Ile Val Met Glu Leu Cys Glu Gly Gly Glu
 30               130                 135                 140

          Leu Leu Asp Arg Ile Leu Ala Lys Lys Asn Ser Arg Tyr Ser Glu Lys
          145                 150                 155                 160

 35       Asp Ala Ala Val Val Val Arg Gln Met Leu Lys Val Ala Ala Glu Cys
                      165                 170                 175

          His Leu Arg Gly Leu Val His Arg Asp Met Lys Pro Glu Asn Phe Leu
 40               180                 185                 190

          Phe Lys Ser Asn Lys Glu Asp Ser Pro Leu Lys Ala Thr Asp Phe Gly
 45               195                 200                 205

          Leu Ser Asp Phe Ile Lys Pro Gly Lys Lys Phe His Asp Ile Val Gly
                  210                 215                 220

 50       Ser Ala Tyr Tyr Val Ala Pro Glu Val Leu Lys Arg Arg Ser Gly Pro
          225                 230                 235                 240

 55       Glu Ser Asp Val Trp Ser Ile Gly Val Ile Thr Tyr Ile Leu Leu Cys
                      245                 250                 255
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      Gly Arg Arg Pro Phe Trp Asp Lys Thr Glu Asp Gly Ile Phe Lys Glu
              260                 265         270

      Val Leu Arg Asn Lys Pro Asp Phe Arg Lys Arg Pro Trp Ser Ser Ile
              275                 280         285

      Ser Pro Gly Ala Lys Asp Phe Val Lys Arg Leu Leu Val Lys Asn Pro
              290                 295         300

      Arg Ala Arg Leu Thr Ala Ala Gln Ala Leu Ser His Pro Trp Val Arg
      305                 310         315                 320

      Glu Gly Gly Glu Ala Ser Asp Ile Pro Val Asp Ile Ser Val Leu Ser
              325                 330         335

      Asn Met Arg Gln Phe Val Lys Tyr Ser Arg Phe Lys Gln Phe Ala Leu
              340                 345         350

      Arg Ala Leu Ala Ser Thr Leu Asn Glu Glu Glu Leu Ser Asp Leu Lys
              355                 360         365

      Asp Gln Phe Asp Ala Ile Asp Ile Asp Lys Ser Gly Ser Ile Ser Ile
              370                 375         380

      Glu Glu Met Arg His Ala Leu Ala Lys Asp Leu Pro Trp Arg Leu Lys
      385                 390         395                 400

      Gly Pro Arg Val Leu Glu Ile Ile Gln Ala Ile Asp Ser Asn Thr Asp
              405                 410         415

      Gly Leu Val Asp Phe Lys Glu Phe Val Ala Ala Thr Leu His Ile His
              420                 425         430

      Gln Met Ala Glu Leu Asp Ser Glu Arg Trp Gly Ile Arg Cys Gln Ala
              435                 440         445

      Ala Phe Ser Lys Phe Asp Leu Asp Gly Asp Gly Tyr Ile Thr Pro Glu
              450                 455         460

      Glu Leu Arg Met Val Gln His Thr Gly Leu Lys Gly Ser Ile Glu Pro
      465                 470         475                 480

      Leu Leu Glu Glu Ala Asp Ile Asp Lys Asp Gly Lys Ile Ser Leu Ser
              485                 490         495

      Glu Phe Arg Lys Leu Leu Arg Thr Ala Ser Met Ser Asn Val Pro Ser
              500                 505         510
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Pro Arg Gly Pro Pro Asn Pro Gln Ala Leu
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<210> 10
<211> 1940
<212> DNA
<213> Zea mays L.

<400> 10

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aaaggcgaca gatttggtt tgtcagattt cattaagcca gggaagaagt tccatgacat     720

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gaatccaagg gccaggctaa cagctgctca agctctctca catccgtggg taagagaagg    1020

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ccggaagagt gagaggtgga ggtcaaaatg gtaggcaaga ctcgccttct tctttccttt   1860

acactgtaca gtgatactga aatatgtacg attttttatta taactgttcg tcgcaataaa   1920

gttatttgga gaagtgagga   1940

<210> 11
<211> 287
<212> DNA
<213> Zea mays L.

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tgttggaagt gcttactatg tcgcaccaga agtactaaaa cgacggtctg gtcctgagtc   180

agatgtttgg agcataggag tcataaccta cattttgctc tgtgggaggc gccctttttg   240

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<211> 13563
<212> DNA
<213> Zea mays L.

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atcagaacat ggtgcagccg catgttggtg atgacaaggg cgatgatgag cggtaatata   120

ccggcccctg ccatgttgtc ggggcagtcg gatgccccaa aagagatagt ggtgctcctc   180

caccgttgat gtggagcagc catcgggacc cccgggaccg ccgaaaggac ctctcggcac   240

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35

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Thr
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Claims

1. A protein, which is as follows: (a1), or (a2), or (a3), or (a4), or (a5), or (a6), or (a7), or (a8):

      (a1) a protein represented by SEQ ID NO: 2 in the sequence listing;
      (a2) a protein represented by SEQ ID NO: 4 in the sequence listing;
      (a3) a protein represented by SEQ ID NO: 7 in the sequence listing;
      (a4) a protein represented by SEQ ID NO: 9 in the sequence listing;
      (a5) a fusion protein obtained by attaching a tag to an N-terminus or/and a C-terminus of the protein in any one of (a1) to (a4);
      (a6) a protein comprising the following three segments from N-terminus to C-terminus: the protein in any one of (a1) to (a4), a connecting peptide, and an EGFP protein;
      (a7) a protein related to plant gray leaf spot resistance obtained by substituting and/or deleting and/or adding one or a plurality of amino acid residues to the protein in any one of (a1) to (a6); and
      (a8) a protein related to plant gray leaf spot resistance obtained from corn and having a homology of 90%, 91%,

92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with the protein in any one of (a1) to (a4).

2. A nucleic acid molecule, which encodes the protein according to claim 1.

3. The nucleic acid molecule according to claim 2, **characterized in that**: the nucleic acid molecule is any one of the following (b1) to (b15):

(b1) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1618 in SEQ ID NO: 3 in the sequence listing;
(b2) a DNA molecule represented by SEQ ID NO: 3 in the sequence listing;
(b3) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1624 in SEQ ID NO: 5 in the sequence listing;
(b4) a DNA molecule represented by SEQ ID NO: 5 in the sequence listing;
(b5) a DNA molecule represented by SEQ ID NO: 1 in the sequence listing;
(b6) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1618 in SEQ ID NO: 8 in the sequence listing;
(b7) a DNA molecule represented by SEQ ID NO: 8 in the sequence listing;
(b8) a DNA molecule with an encoding region that is represented by nucleotides 56 to 1624 in SEQ ID NO: 10 in the sequence listing;
(b9) a DNA molecule represented by SEQ ID NO: 10 in the sequence listing;
(b10) a DNA molecule represented by SEQ ID NO: 6 in the sequence listing;
(b11) a DNA molecule represented by SEQ ID NO: 12 in the sequence listing;
(b12) a DNA molecule represented by SEQ ID NO: 13 in the sequence listing;
(b13) a DNA molecule represented by SEQ ID NO: 14 in the sequence listing;
(b14) a DNA molecule that is derived from corn, has a homology of 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with any one of (b1) to (b13), and encodes the protein;
(b15) a DNA molecule that hybridizes to any one of (b1) to (b13) under a stringent condition, and encodes the protein.

4. An expression cassette, a recombinant vector or a recombinant microorganism comprising the nucleic acid molecule according to claim 2 or 3.

5. An application of the protein according to claim 1, which is the following (c1) or (c2):

(c1) to regulate the resistance of a plant to gray leaf spot;
(c2) to reduce the disease resistance of a plant to gray leaf spot.

6. The application according to claim 5, **characterized in that** the plant is a plant of the genus Zea.

7. An application of the nucleic acid molecule according to claim 2 or 3, which is the following (d1) or (d2):

(d1) to cultivate a transgenic plant with altered resistance to gray leaf spot;
(d2) to cultivate a transgenic plant with reduced resistance to gray leaf spot.

8. The application according to claim 7, **characterized in that** the plant is a plant of the genus Zea.

9. An application of a substance for inhibiting an activity of the protein according to claim 1 in a plant and/or for reducing an abundance of the protein according to claim 1 in a plant to enhance the disease resistance of the plant to gray leaf spot.

10. The application according to claim 9, **characterized in that** the plant is a plant of the genus Zea.

11. An application of a substance for inhibiting a transcription of the nucleic acid molecule according to claim 2 or 3 and/or for inhibiting an expression of the nucleic acid molecule according to claim 2 or 3 and/or for gene editing of the nucleic acid molecule according to claim 2 or 3 to enhance the disease resistance of the plant to gray leaf spot.

12. The application according to claim 11, **characterized in that** the plant is a plant of the genus Zea.

13. A method for preparing a transgenic plant, comprising the steps of: introducing the nucleic acid molecule according to claim 2 or 3 into a starting plant to obtain a transgenic plant with reduced gray leaf spot resistance.

14. The application according to claim 13, **characterized in that** the starting plant is a plant of the genus Zea.

15. A plant breeding method, comprising the following steps: increasing a content and/or activity of the protein according to claim 1 in a target plant, thereby reducing the gray leaf spot resistance of the target plant.

16. The application according to claim 15, **characterized in that** the target plant is a plant of the genus Zea.

17. A method for preparing a transgenic plant, comprising the following steps: inhibiting an expression of the nucleic acid molecule according to claim 2 or 3 in a starting plant to obtain a transgenic plant with increased gray leaf spot resistance.

18. The application according to claim 17, **characterized in that** the starting plant is a plant of the genus Zea.

19. A plant breeding method, comprising the following steps: reducing a content and/or activity of the protein according to claim 1 in a target plant, thereby increasing the disease resistance of the target plant to gray leaf spot.

20. The application according to claim 19, **characterized in that** the target plant is a plant of the genus Zea.

21. A plant breeding method, comprising the steps of: performing gene editing on a specific gene in a genome of a starting plant to increase the gray leaf spot resistance of the target plant; and the specific gene encoding the protein according to claim 1.

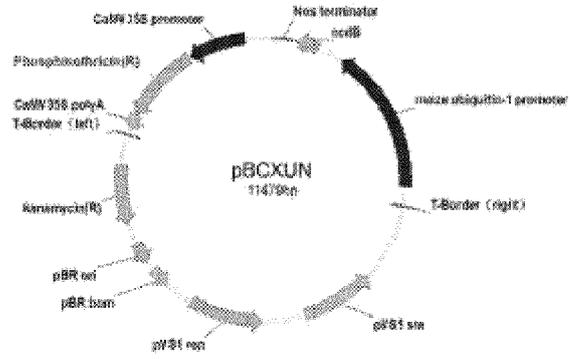22. The application according to claim 21, **characterized in that** the starting plant is a plant of the genus Zea.
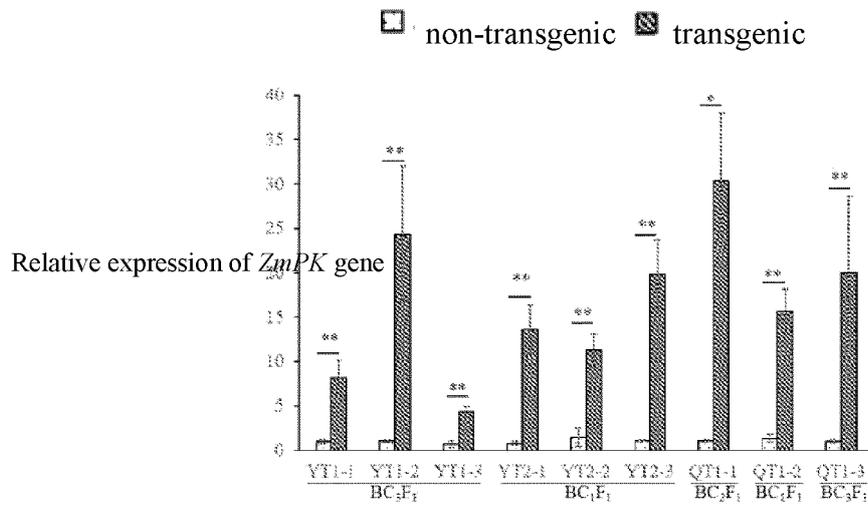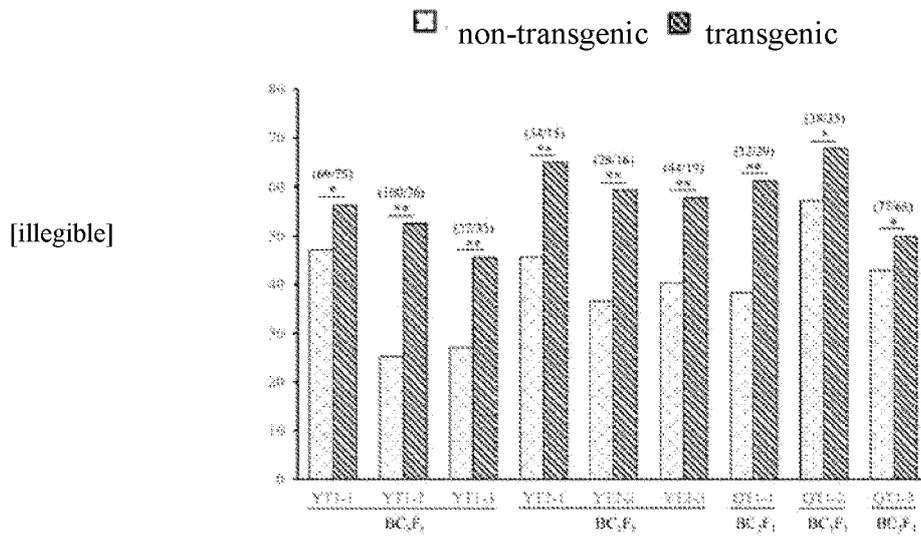
Fig. 1

Fig. 2

[illegible]

Fig. 3

HindIII   EcoRI   BamHI   XbaI

35S   GUS   CaMV
ter

Fig. 4

☐ non-transgenic ▨ transgenic

Relative expression of *ZmPK* gene

Fig. 5

☐ non-transgenic ▨ transgenic

Fig. 6

$R^2 = 0.97$

Relative expression of *ZmPK* gene

Disease level

Fig. 7

Fig. 8

Relative expression of ZmDR gene

non-transgenic

transgenic

Fig. 9

non-transgenic ▨ transgenic

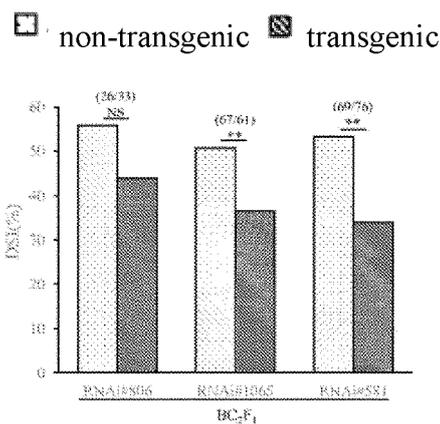Fig. 10

Fig. 11

Relative expression of *ZmPK* gene
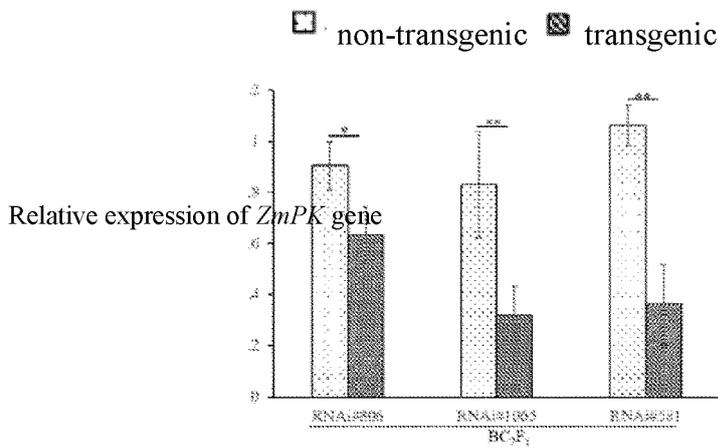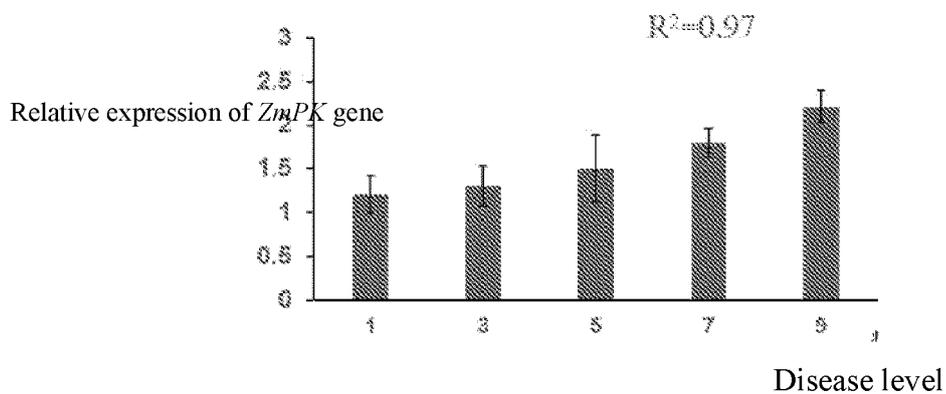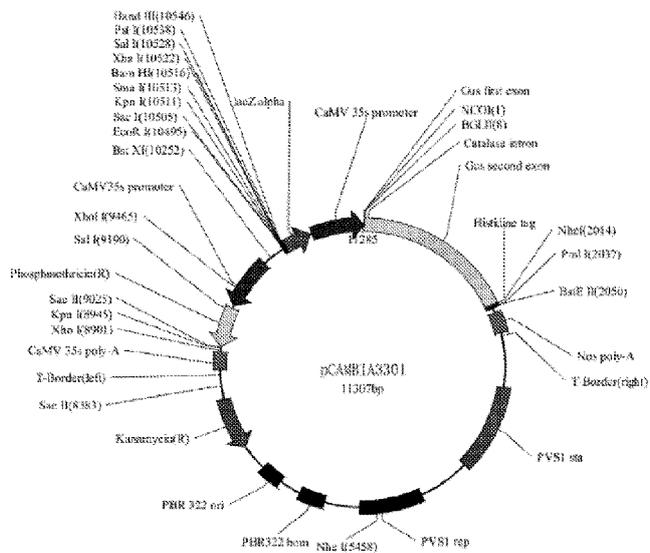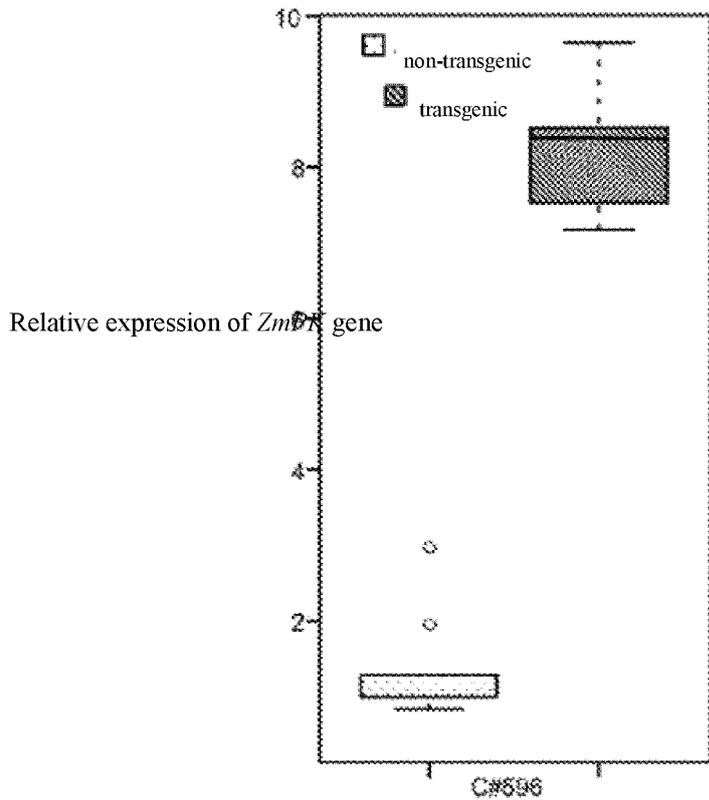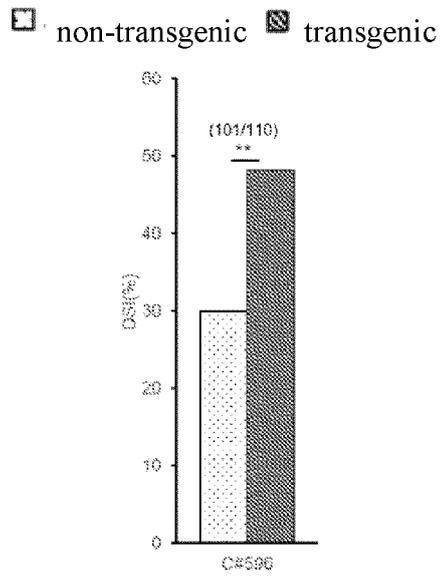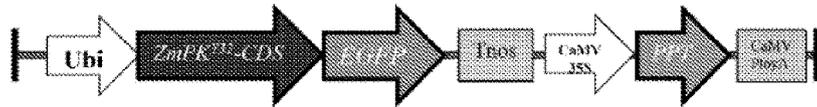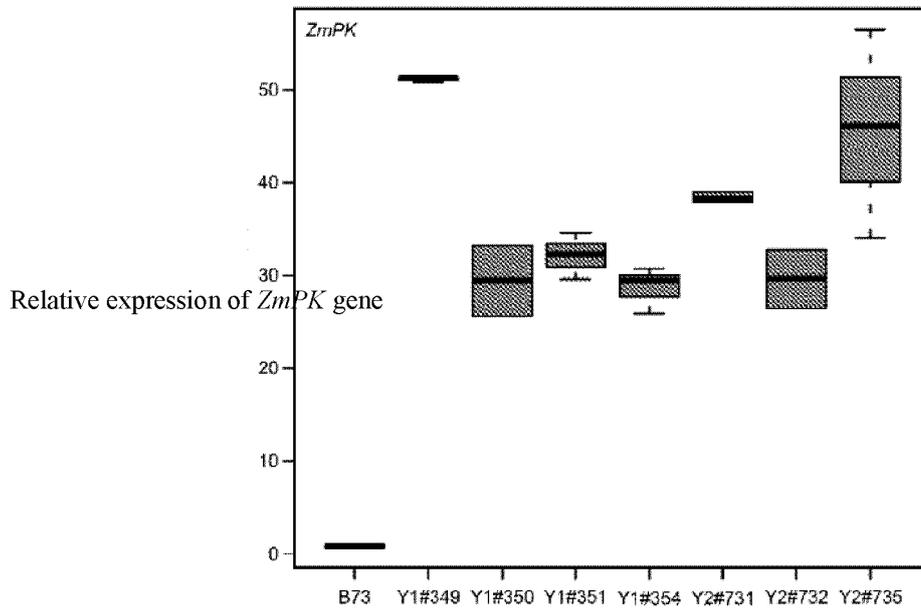
Fig. 12

Fig. 13

Fig. 14

Fig. 15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2020/076319

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/415(2006.01)i; C12N 15/29(2006.01)i; C12N 15/82(2006.01)i; A01H 5/00(2018.01)i; A01H 6/46(2018.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; C12N; A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNKI: 玉米, 灰斑病, 钙依赖性蛋白激酶, maize, Zea mays, gray leaf spot, GLS, qRgls2, GRMZM2G157068, 等; Genbank: SEQ ID NO: 2-9

C. DOCUMENTS CONSIDERED TO BE RELEVANT

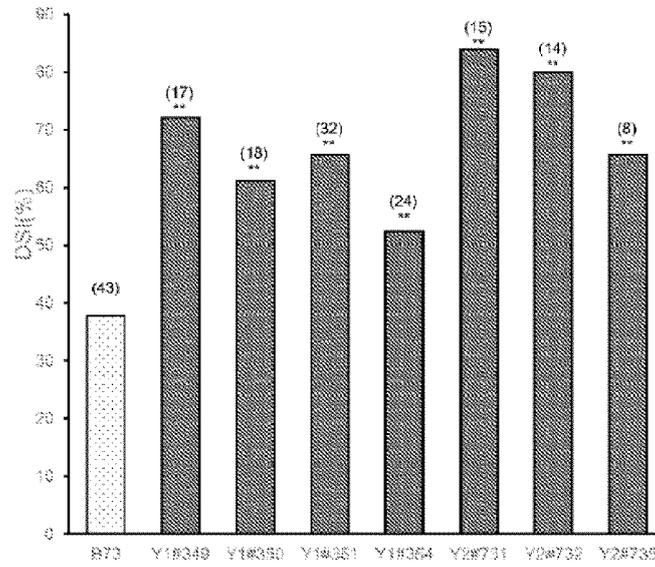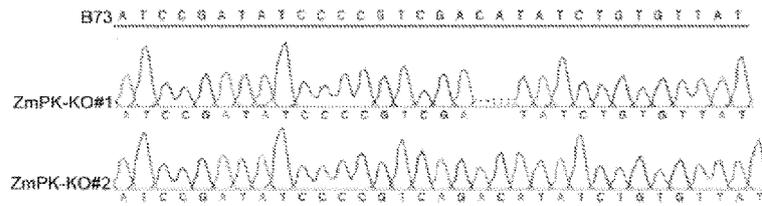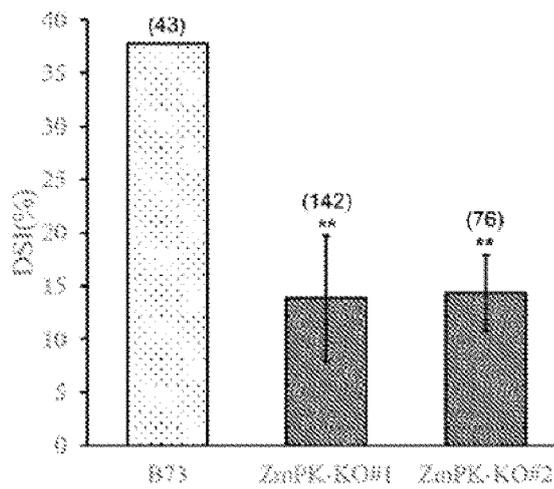| Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	CN 109705202 A (CHINA AGRICULTURAL UNIVERSITY) 03 May 2019 (2019-05-03) claims 1-10	1-22
X	GenBank. "Version NM_001359579.1" *GenBank Registration No. NM_001359579*, 05 May 2018 (2018-05-05), DEFINITION, FEATURES and ORIGIN parts	1-4
Y	GenBank. "Version NM_001359579.1" *GenBank Registration No. NM_001359579*, 05 May 2018 (2018-05-05), DEFINITION, FEATURES and ORIGIN parts	5-22
Y	徐凌 (XU, Ling). "玉米抗灰斑病主效QTL精细定位、基因预测及转录组分析 (Fine-mapping a Major QTL Resistance to Gray Leaf Spot in Maize, Gene Prediction and Transcriptome Analysis)" 中国博士学位论文全文数据库 (*China Doctoral Dissertations Full-Text Database*), 10 June 2018 (2018-06-10), abstract, page 34, section 3.2, table 3.1, and page 57, section 5.3	5-22
A	WO 2009088756 A1 (DU PONT et al.) 16 July 2009 (2009-07-16) entire document	1-22

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	
"E" earlier application or patent but published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 May 2020	**03 June 2020**

Name and mailing address of the ISA/CN	Authorized officer
China National Intellectual Property Administration (ISA/CN) **No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088 China**	
Facsimile No. **(86-10)62019451**	Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2020/076319

C. **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 106701972 A (HUAZHONG AGRICULTURAL UNIVERSITY; HUBEI TENGLONG SEED CO., LTD.) 24 May 2017 (2017-05-24) entire document	1-22
A	CN 101970669 A (E. I. DU PONT DE NEMOURS AND COMPANY; PIONEER HI-BRED INTERNATIONAL, INC.) 09 February 2011 (2011-02-09) entire document	1-22
A	WO 2018013323 A1 (PIONEER HI-BRED INT INC) 18 January 2018 (2018-01-18) entire document	1-22

Form PCT/ISA/210 (second sheet) (January 2015)

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INTERNATIONAL SEARCH REPORT

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International application No.
PCT/CN2020/076319

Box No. I   Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

a. ☑ forming part of the international application as filed:

☑ in the form of an Annex C/ST.25 text file.

☐ on paper or in the form of an image file.

b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2020/076319

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	109705202	A	03 May 2019	None			
WO	2009088756	A1	16 July 2009	CN	101970669	B	12 June 2013
				BR	PI0819551	A2	10 March 2015
				US	8841509	B2	23 September 2014
				CN	101970669	A	09 February 2011
				EP	2653549	A1	23 October 2013
				US	2013177907	A1	11 July 2013
				ZA	201004402	B	31 August 2011
				US	9150933	B2	06 October 2015
				US	2015074841	A1	12 March 2015
				EP	2240588	A1	20 October 2010
				BR	PI0819551	B1	19 December 2017
				CA	2710529	C	29 August 2017
				MX	2010007258	A	16 August 2010
				EP	2653549	B1	30 March 2016
				CN	103477992	B	29 July 2015
				US	2009172845	A1	02 July 2009
				CN	103477992	A	01 January 2014
				US	8367899	B2	05 February 2013
				CA	2710529	A1	16 July 2009
				EP	2240588	B1	08 April 2015
CN	106701972	A	24 May 2017	CN	106701972	B	16 July 2019
CN	101970669	A	09 February 2011	CN	101970669	B	12 June 2013
				BR	PI0819551	A2	10 March 2015
				US	8841509	B2	23 September 2014
				EP	2653549	A1	23 October 2013
				US	2013177907	A1	11 July 2013
				ZA	201004402	B	31 August 2011
				US	9150933	B2	06 October 2015
				US	2015074841	A1	12 March 2015
				EP	2240588	A1	20 October 2010
				BR	PI0819551	B1	19 December 2017
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				US	2009172845	A1	02 July 2009
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				US	8367899	B2	05 February 2013
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				EP	2240588	B1	08 April 2015
				WO	2009088756	A1	16 July 2009
WO	2018013323	A1	18 January 2018	CN	109688805	A	26 April 2019
				BR	112019000579	A2	02 July 2019
				EP	3481176	A4	12 February 2020
				CA	3029819	A1	18 January 2018
				EA	201990256	A1	31 July 2019
				US	2019300969	A1	03 October 2019
				ZA	201900149	B	28 August 2019
				EP	3481176	A1	15 May 2019

Form PCT/ISA/210 (patent family annex) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

Patent document cited in search report	Publication date (day/month/year)	Patent family member(s)	Publication date (day/month/year)

REFERENCES CITED IN THE DESCRIPTION

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Non-patent literature cited in the description

- **YAN ZHANG.** *Theor Appl Genet* **[0032] [0033]**
- **PATRICK S. SCHNABLE.** *Science,* 2009, vol. 326, 1112-1115 **[0034]**
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